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† In the order of presentation at the meeting

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ANNUAL MEETING

COUNCIL FOR HIGH BLOOD PRESSURE RESEARCH

Scientific Reports

YALE J KATZ M D Ph D —Department of Medicine University of Southern California Los Angeles Calif

ABBIE I KNOWLTON M D —Department of Medicine Columbia University College of Physicians and Surgeons and the Presbyterian Hospital New York N Y

JEAN OLIVER M D —Renal Research Unit Overlook Hospital Summit N J

DANIEL C PEASE Ph D —Department of Anatomy School of Medicine University of California Los Angeles Calif

FLOYD R SKELTON M D Ph D —Urban Mae Research Foundation and Department of Pathology Louisiana State University Medical School New Orleans La

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STUDIES ON THE HYPERTENSIVE ACTION OF ADRENAL STEROIDS

ABBIE I KNOWLTON, M D , *Assistant Professor of Medicine, Department of Medicine, Columbia University College of Physicians and Surgeons, and the Presbyterian Hospital, New York, N Y*

For a number of years Dr Emily N Loeb Dr Herbert C Stoerk and myself have been interested in the relationship between the adrenal steroids and hypertension and we have all shared in the work that I shall report In earlier studies Dr Beatrice C Seegal joined us and along the way we have had considerable assistance from medical students who spent the elective period of their third year in our laboratory and who made many valuable contributions to our work

I shall not attempt to summarize the literature on the subject of the adrenals and hypertension for you are perhaps better acquainted with this than I am I shall confine myself to reviewing our own studies

A basic question which remains in considering this problem is whether hypertension of *any* type can exist in the absence of the adrenal glands or its hormones This question has been answered variously in the literature but in our experience the answer is Yes at least in the rat(1) Such a hypertensive animal is shown in Figure 1 This rat, representative of a series developed a significant rise in tension after renal damage had been produced by the intravenous injection of an antirat kidney serum In the upper portion of the chart the weekly blood pressure readings on this animal are plotted In these and earlier studies the plethysmographic apparatus described by Williams Harrison and Grollman(2) was used in making the determinations After Friedman and Freed(3) described their method involving the use of the microphonic manometer we shifted to this technique but did not curarize the animals In the lower part of the chart is plotted the weight of the rat from week to week The antikidney serum was injected 18 days after bilateral adrenalectomy and a high sodium regimen was instituted i e a diet made up of 1 7 per cent sodium chloride and normal saline provided in place of tap water Following the injection of the renal damaging agent, hypertension developed In order to confirm the completeness of the adrenalectomy the animal was subsequently at the point indicated by the arrow placed upon a sodium restricted regimen The rapid weight loss dramatic fall in blood pressure and death which ensued afforded convincing evidence of a lack of residual adrenal tissue

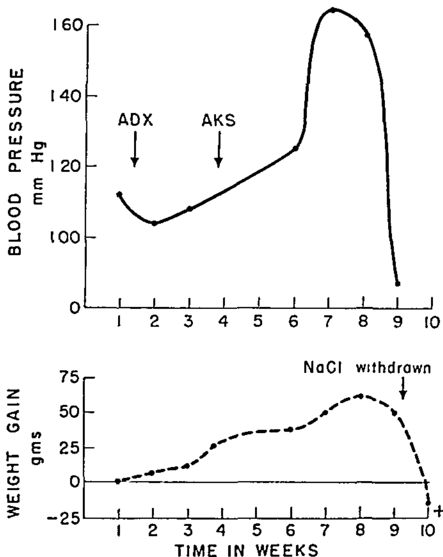


FIGURE 1 Changes in blood pressure and weight in an adrenalectomized rat given antikidney serum. Reprinted from Knowlton A. I., Loeb E. N., Seegal B. C., Stoerk, H. C., and Berg J. L. Development of hypertension in adrenalectomized nephritic rat maintained on NaCl Proc Soc Exper Biol and Med 74 661 1950

It would seem therefore that hypertension at least of certain types can develop in the adrenalectomized rat given no steroid replacement therapy

A second and quite separate question is whether the individual adrenal steroids may under certain condition be hypertensive agents The answer to this is again Yes This was first demonstrated for the steroid desoxycorticosterone (DCA) by Kuhlmann et al (4) Subsequently Selye (5) pointed out that an experimental animal could be sensitized to the hypertensive action of this compound if the renal mass were reduced if the kidneys were damaged or if a high sodium intake were furnished

The relationship between the sodium ion and DCA is a particularly interesting one since the so called DCA hypertension can develop only when sufficient quantities of this ion are available In other words this form of hypertension can be wholly prevented by rigid restriction of the intake of this electrolyte even in animals sensitized by renal damage(6)

Figure 2 shows graphically some data which illustrate this point This experiment was designed so that the sodium intake which constituted 1 5 per cent of the diet as sodium chloride did not significantly alter the blood pressure either by itself (see the fourth group on the upper line) or in combination with DCA in a dosage of 2 5 mg daily (second group on upper line) or with the renal damaging agent which was a rabbit antirat kidney serum (last group in lower line) However a combination of these three factors as anticipated led to a striking rise in arterial tension (second group on lower line) Of particular interest is the first group on the lower line in which the omission of sodium entirely prevented the hypertension which marked the group provided with liberal quantities of this cation In addition to averting the appearance of hypertension the rats on this low sodium intake failed to develop other characteristic signs of excess DCA action i e they showed no renal or cardiac hypertrophy and even the histological evidences of DCA activity were minimized

The group shown third from the left in the upper line and also the group in a similar position in the lower line were included in this experiment to determine whether the addition of a potassium chloride supplement would prevent the appearance of DCA hypertension This possibility was considered since it was known that such a supplement could prevent the abnormal increase in skeletal muscle sodium noted in DCA dogs It seemed possible that DCA hypertension was in some way related to the accompanying electrolyte shifts However the data here indicate that the addition of potassium in no way minimized the rise in blood pressure although the serum electrolytes in the potassium treated groups were less abnormal than in the DCA groups receiving only sodium chloride Subsequently Rosenman Freed and Friedman(7) have reported that a liberal intake of potassium permits the prolonged main

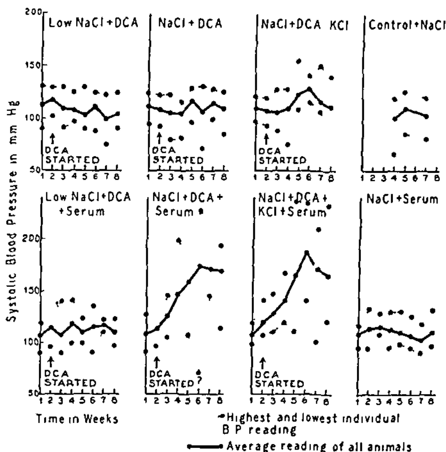


Figure ? *Effect of DCA NaCl KCl and antikidney serum on rat blood pressure* Reprinted from Knowlton A I., Loeb E N., Stoerk H C., and Seegal B C. Desoxycorticosterone acetate the potentiation of its activity by sodium chloride J Exp r Med 85 18, 1947

tenance of DCA hypertension whereas a high sodium diet alone may not due to the development in the latter instance of potassium deficiency

Certain other steroids behave similarly to DCA in that their hypertensive properties are accentuated by sensitizing the experimental animal through a reduction of renal mass in the presence of high sodium intake Within the past year Friedman Friedman and Nakashima(8) and also Selye(9) have reported that 9 α chlorohydrocortisone resembles DCA in this regard As with DCA it is also possible to prevent the development of hypertension with this halogenated hydrocortisone by the restriction of sodium chloride provided that a relatively low dosage of the compound is selected(10) Figure 3 sets forth data on this point All these animals were sensitized at the beginning of the experiment by unilateral nephrectomy half were maintained on a diet restricted to 0 02 per cent or less of sodium and half were offered a high sodium diet, 0 6 per cent and given physiological saline to drink in place of tap water One mg of DCA was injected subcutaneously for six days weekly this being a known pressor dose of the steroid To provide a comparable degree of sodium retention the dosage of 9 α chlorohydrocortisone acetate was set at 0.2 mg Additional experimental groups were injected with 1 mg DCA and 0 6 mg of hydrocortisone acetate the latter calculated to supply a degree of activity as measured in liver glycogen assays equal to the 0 2 mg of 9 α chlorohydrocortisone acetate From Figure 3 it is evident that severe and equal degrees of hypertension developed in all three experimental groups maintained on a high sodium regimen That this blood pressure effect was virtually dependent upon the liberal sodium provided is proven by the absence of hypertension in the similarly injected groups in which sodium was restricted

Although we have had no experience with aldosterone it might be mentioned that this compound also exhibits a DCA like effect upon the blood pressure Initially Gross Loustalot and Meier(11) were unable to evoke hypertension with this most potent natural adrenal steroid but Kumar Anderson and Gornall(12) have more recently reported the induction of hypertension cardiac and renal hypertrophy with prolonged administration of the compound

The next question to be answered is whether those adrenal steroids with a relatively slight capacity to retain sodium are also hypertensive agents and if so under what conditions In our experience and in that of the Friedmans and Nakashima(13) the answer to the first part of this question is Yes The conditions under which hypertension occurs however are less limited than is seen with DCA in that it is wholly independent of the sodium content of the diet(14)

AVERAGE BLOOD PRESSURE READINGS

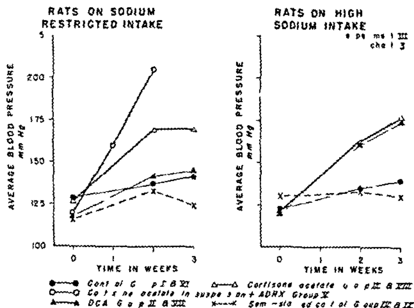


Figure 4 Comparison of the effect of cortisone and DCA upon rat blood pressure Reprinted from Knowlton A I Loeb E N, Stoerk H C White J P, and Heffernan J F Induction of arterial hypertension in normal and adrenalectomized rats given cortisone acetate J Exper Med 96 1952

Figure 4 illustrates this fact In this experiment the effects of cortisone were compared to those of DCA in *normal* rats maintained either on sodium restriction or on a high sodium intake Two groups of untreated control animals were also observed on the experimental diets one of these eating *ad lib* and the other offered a limited food intake so that the ensuing weight loss resulted in body weights approximating that of the cortisone injected rats In the groups given liberal quantities of sodium both the steroid injected groups behaved similarly and developed significant rises in blood pressure in contrast to both uninjected groups The difference between the two steroids became apparent in the groups on sodium restriction Here the cortisone treated animals were as hypertensive as similar rats on a high sodium intake although the DCA rats under these conditions showed no more rise in tension than did the uninjected controls A group of adrenalectomized animals was included and in this instance it appeared that the effect of cortisone was even more marked in the absence of the adrenal gland This has not however proven so in a subsequent study in which the hypertensive activity of the compound had been of equal degree with and without the adrenals *in situ* Besides cortisone Friedman Friedman and Nakashima(13) have reported that hydrocortisone is a hypertensive agent and this has been our experience also

Table I

Incidence of Hypertension in Sodium Restricted Adrenalectomi ed Rats Injected with Steroids

Daily steroid dosage	Intact controls	Hydrocortisone Cortisone	1—dehydro—F 1—dehydro—E	9 α—FIF	9 α—CIF	1—dehydro—9 α—FIF	DOC
mg 0	1/38#						
0 008						0/5***	
0 01				0/3 **	0/8		
0 03				0/8		0/8	all*
0 1		all*			0/8		0/8
0 12						10/32	0/4****
0 2		all*		1/7*			
0 3		all*					
0 33				12/24			
0 4						2/6**	
0 5		all*	all*				
0 8			6/19*****				
1 0		7/25	1/11*****	13/25****	5/8	all*	0/7*
1 2						0/1* *****	
2 0		5/7*	3/7*				
2 5		28/30		1/4 **			
3 0		6/6 *					0/8

Numerator denotes number of rats with blood pre sure in exce s of 165 denominator denotes total number of rats examin d
Each star represents a death or an unobtainable blood pressure reading
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Within the last few years a number of synthetic steroids have been prepared all possessing remarkable physiological activity with respect to liver glycogen assays although with widely varying degrees of activity with respect to sodium-retaining capacity. We have recently explored the effect of a number of these compounds upon the blood pressure in comparison with hydrocortisone, cortisone and DCA (15). Adrenalectomized rats were employed and a sodium-poor diet was selected in order to avoid any facilitation of a DCA-like hypertension such as was demonstrated above with 9 α-chlorohydrocortisone. Table I sets forth the incidence of hypertension, the numerator giving the number of rats with readings in excess of 165 mm Hg and the denominator the number of animals in which blood pressures were obtained at the stated dosage. The data pertaining to cortisone and hydrocortisone being indistinguishable have been combined in the tables and figures; for similar reasons the data on 1-dehydro-hydrocortisone and 1-dehydrocortisone have been presented jointly.

As had been previously noted the hypertensive action of hydrocortisone and of cortisone was again evident in the higher dosages and at the 2.5 and 3 mg dosage levels an overwhelming percentage of the rats were hypertensive. At appropriate dosage levels each of the several synthetic compounds studied resulted in the appearance of elevated readings in a significant percentage of the animals but it is evident from the averages shown in Table II that none led to as severe a degree of hypertension consistently as did hydrocortisone or cortisone. In the capacity to induce hypertension under these sodium-restricted conditions all these compounds behave like hydrocortisone rather than like DCA since this latter steroid even in doses as high as 3 mg daily on the salt-free diet employed did not result in hypertension in a single animal. Our initial thought had been that the hypertensive property of cortisone under these sodium-restricted conditions was linked in some manner to that activity which is measured in liver glycogen assays. This would be consistent with the failure of DCA to produce hypertension with the regimen as this steroid is inactive in this particular type of assay.

At Dr Stoerk's suggestion log dose curves of blood pressure were computed for hydrocortisone, DCA and for each of the analogues shown except 1-dehydro-hydrocortisone and 1-dehydrocortisone. Here the dosage range which permitted survival was too narrow to provide the necessary data to compute a line.*

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* For statistical advice in these analyses we are indebted to Dr. John W. Fertig and Miss E. B. [illegible] of the School of Public Health and Administrative Medicine of the Faculty of Medicine, Columbia University, New York, N. Y.

TABLE II

Eff t f St d Blood I of 4d l t m d R ts S d m R t t

D t st roid dosage	I t on t	Hyd or rt sone (so	1—d hyd o—F 1—d h d o—E	9-a—FHF	9-a—ClF	1—d hy l o— 9-a—FHF	DOC
mg 0	115±12 7(38)#						
0 008						101±18 1(5)	
0 01				66±21 (3)	121±13 (8)		
0 03				10 ±20 8(8)		120±12 1(8)	||
0 1		||(8)			119± 9 0(8)		100± 8(8)
0 12						153±11 0(8) 165± 9 9(8) 171±13 1(8) 158± 9 2(8)	99±17 (4)
0 2		||(8)		163±11 3(7)			
0 3		||(8)					
0.33				157±14 1(8) 1 6±11 6(8) 161±10 2(8)			
0 4						160±12 9(6)	
0 5		||(8)	||(8)				
0.8			101± 8 7(4) 1 3±13 (8) 149±17 0(9)				
1 0		1 2± 7 6(4)## 156±23 3(8) 16(±16 1(5) 137± 8 3(8)	130± 2 8(3) 151±10 8(8)	166±15 6(5) 1 6± 2 8(7) 13 ±15.8(6) 166±12 7(7)	169± 9 6(8)	||	1 0±11 8(7)
1.2						138(1)	
2 0		177±18 1(8)	159±21 0(7)				
2.5		18 ±15.2(8) 192±?0 8(6) 181± 8 8(8) 181±15 7(8)		148±21 9(4)			
3 0		189±10 6(6)					150± 7 1(8)

Ea h fig re represe he ve ge f blood pressure re d gs f 1 38 d d in p ren heses St d d dev so h h f ll g det m d

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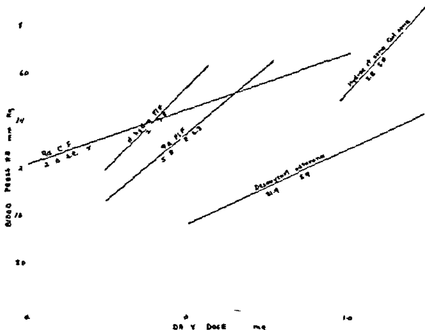


FIGURE 5 Effect of analogues of hydrocortisone on blood pressure of adrenalectomized rat. From Knowlton, A. I., Loeb, E. N. and Stoerk, H. C. Effect of synthetic analogues of hydrocortisone on the blood pressure of adrenalectomized rats on sodium restriction. To be published in Endocrinology

Figure 5 presents these log dose curves and the slope of 73.8 for the hydro*cortisone and cortisone line is significantly different statistically from the* slope of 31.9 for DOC. Were it true that the blood pressure elevating activity of the several analogues varied directly with liver glycogen activity then the line of each should parallel the hydrocortisone line while if the blood pressure elevating activity varied with the capacity to retain sodium each should parallel the DOC line. Neither hypothesis fits the observed findings that the slopes of the lines for *9α fluorohydrocortisone* and *1 dehydro 9α fluoro*hydrocortisone do not differ significantly from that of the hydrocortisone line while the slope of *9α chlorohydrocortisone* resembles that of DOC. *It will be recalled that the two fluorinated compounds differ from the chlorinated* compound in that the ratio of the activity as measured by the liver glycogen assay to the activity with regard to sodium retention is relatively higher (see Table III in which data relating the activity of the several steroids as com

TABLE III

Relatiie Actiuty of Synthetic Steroids Compared to Cortisone and DOC

Compound	CORTISONE E / HYDROCORTISONE F	DESOXYCORTICOSTERONE DOC
P..D..L..O.. 1 .. O E	4	v ry slight
PR D .. O.. 1 ..D..O F		
9α-F.. LO C- ..D.. O ..TISO.. 9α F1F	10 t 1	1 t 5
1 .. C ..-.. O C .. OC TI C..	5 to ?0	5
9α-C.. CO .. 9α-Cl..	3 to ?	2 to ?0

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pared to hydrocorti one and de oxycortico terone has been gathered from the literature) This gave ri e to the hypothe is that the blood pressure elevating the effect of the e teroid was dependent on neither of the e two adrenal activitie alone but in tead was dependent on the ratio of the two present in the compound

This po sibility was pursued with an attempt to reproduce the lope of 9 a chlorohydrocorti one by a combination of 1 dehydro hydrocorti one and DOC in which the relative proportions of liver glycogen to odium retaining activities duplicated tho e exi ting in the chloro compound Examination of Figure 6 reveals that the attempt wa highly succe ful the lope of the two lines being indi tingui hable although each howed a ugge tion of a lack of linearity

The e ob ervations till leave unknown the mechanisms by which the adrenal steroids evoke a ri e in blood pre sure although one may conclude that different mechani ms operate in the ca e of DCA and of corti one In an

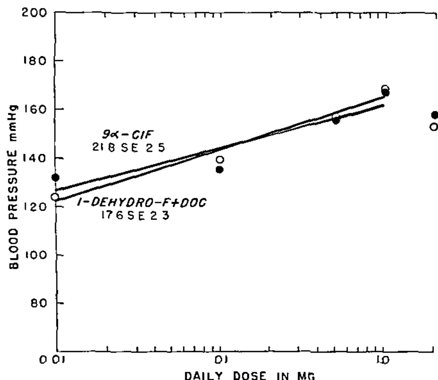


FIGURE 6 Log dose r pon e 9-α ClF s 1-dehydro-F + DOC Fr n Kn wlton A I Loeb E. N and Stoerk H C Effect of synthet c analogues of hyd ocort sone on th bl od p ssu e of lr i alect nu l rats on o l um r t icti n To le p b l sl ed in Endocrinology

TABLE IV

Body Weight Blood Pressure and Carcass Composition of Rats After Two Weeks of Restricted Sodium Intake

Group	Final body weight (Gm)	Final blood pressure mm Hg	Carcass analysis						
			Water %	Fat % DT[1]	Na	K	Cl	N	
					mEq/100 Gm FFDT[2]			% FFDT[2] Gm	% FFWT[3] Gm
Normals	160	112	66.7	20.1	15.3	25.0	10.2	10.8	3.03
ADRX	113*	81*	70.0	11.0	13.8	25.5	10.8	11.3	3.17
ADRX + DCA	166	128	68.6	14.1	11.9	23.6	10.6	9.9	2.66
ADRX + E	103*	10[illegible]*	65.2	11.9	15.1	21.1*	9.4	10.2	3.25
Normals on limited intake	111*	112	69.5*	15.4	17.0	21.9	11.7	11.0	3.09

[1] Dried tissue

[2] Fat free dried tissue

[3] Fat free wet tissue

* Significantly different from "normal" values P = 0.05

Unpublished data

attempt to characterize further the difference between DCA and cortisone hypertension we have undertaken tissue analyses of electrolyte content on pooled tissue of groups of rats. Currently this study has been repeated analyzing each rat individually (16). Many studies are available indicating that DCA results in marked alterations in the composition of skeletal muscle, an increase in the intracellular content of sodium and a decrease in potassium being characteristically found (17). Other tissues have shown changes less consistently. A few studies are available for cortisone, the changes in dogs being less consistent but in the same direction as those seen with *DCA* (18). Rather than analyze individual tissues we elected to determine the electrolyte content of the total carcass, although we recognized that this approach might not detect subtle changes limited to a single tissue such as increases in the arterial tissue as reported by Tobian (19) and that even significant changes in one tissue might be masked by opposing changes in another. However if a major difference in the effect of the two steroids existed, it seemed conceivable that it might be reflected in the total body composition.

The carcasses analyzed were those of adrenalectomized rats which had been injected with 2.5 mg. of DCA or cortisone daily for a two- to three-week period. The effects of these compounds on sodium restricted regimen as well as under conditions of high sodium intake were explored and appropriate control groups included. At sacrifice the animals were exsanguinated and the gastrointestinal tract was removed along with the heart, kidneys, right femur and gastrocnemius, and a 1.2 cm. of skin. On the remaining carcass water content was calculated from the weight loss of 20 Gm. aliquots of ground tissue after a two-week period of desiccation in an electric oven at 90 to 105° C. The dried tissue from two to four individual animals was pooled and the fat content determined by ether extraction in a Soxhlet's apparatus. The defatted tissue was then finely ground by hand in a mortar and triplicate determinations of sodium, potassium and chloride were carried out on 0.5 Gm. samples and of nitrogen on 1.0 Gm. samples.

Table IV presents the data obtained in the rats maintained on a sodium restricted intake under which circumstances the cortisone but not the DCA injected rats became hypertensive. In the first two columns terminal body weights and blood pressures are given. Initial weight and blood pressure in all groups had been comparable averaging 129.134 Gm. Adrenalectomy without steroid replacement therapy led to rapid weight loss and fall in blood pressure and necessitated the sacrifice of this group after four to six days because of their moribund condition. The body weight of this group has been marked with an asterisk and throughout this and the succeeding two tables determinations varying significantly from the normal have been so indicated.*

* For statistical advice in these analyses we are indebted to Dr. Agnes P. Berger and Miss E. Berquo of the School of Public Health and Administrative Medicine of the Faculty of Medicine, Columbia University, New York, N. Y.

TABLE V

Body Weight Blood Pressure and Carcass Composition of Rats After Three Weeks of High Sodium Intake

Group	Final body weight Gm	Final blood pressure mm Hg	Carcass analysis						
			Water %	Fat % DT[1]	Na mEq/100	K Gm	Cl FFDT[2]	N % FFDT[2] Gm	N % FFWT Gm
Normals	165	155	67 3	20 7	17 6	25 5	11 9	11 4	3 19
ADRX	163	126	71 5*	7 7	16 6	21 7	13 6	11 0	2 95
ADRX + DCA	168	172	68 8	18 9	21 2*	22 3	13 5	11 2	3 00
ADRX + DCA + KCl	167	175	70 2*	19 1	19 1	23 7	14 9*	11 1	2 97
[illegible]	108*	167	65 8	17 5	17 5	23 4	12 5	10 5	3 13

[illegible]ed tissue.

[illegible] e wet

S[illegible] ormal values, P = 0 05

The two adrenalectomized groups injected with steroid survived the experimental period of two weeks those on DCA growing as well as the normals The terminal weights of the cortisone treated rats emphasize the impressive growth suppression which this compound exerts in the rat at the dosage level of 2.5 mg daily The final group were included to examine the effects of a similar degree of weight loss per se

Of interest in the carcass analyses were the findings in the hypertensive cortisone injected rats The water content of this group was significantly less than that of normal animals starved to a similar weight and the potassium content was strikingly reduced The remaining determinations in this group did not differ from normal The DCA injected rats which on the sodium restricted regimen remained normotensive showed no variation from normal in the composition of carcasses

Turning to the groups maintained on a high sodium regimen (Table V) it is evident that on this regimen the adrenalectomized rat is able to grow normally without steroid supplements However the weight loss of the cortisone injected animals remained as striking as on the low sodium regimen Both DCA injected groups as well as the cortisone group exhibited blood pressures significantly higher than those of the adrenalectomized control although not higher than those of the normals which in this series were slightly elevated

Also of interest in the carcass analyses were the findings in the two groups of DCA injected hypertensive rats Here the impressive changes from normal were the increases in body water in the rats given a potassium supplement and in carcass sodium in the other DCA group Since equally impressive increases in body water occurred in the normotensive adrenalectomized controls and since the addition of a potassium supplement prevented the accumulation of excess amounts of sodium without diminishing the blood pressure it is difficult to be certain of the relationship of these changes to the DCA hypertension In contrast to the DCA injected group the rats receiving cortisone did not show any increase in body water or in sodium The reduction in potassium noted in the low sodium groups was not impressive in these animals on a high sodium regimen

These tissue analysis studies demonstrate differences between the effects of DCA and cortisone in the rat DCA when accompanied by liberal sodium leads to an abnormal increase in the total body content of this ion With cortisone neither of the experimental regimens led to an increase in total body sodium Despite this lowering of the potassium did occur and most markedly in the sodium restricted animals which in this particular study were relatively more hypertensive than the group on high sodium One might hypothesize that with DCA the primary action of the compound is to increase sodium retention while with cortisone it is the potassium decrease which is the primary one

TABLE VI

Body Weight Blood Pressure and Carcass Composition of Rats with Renal Damage

Regimen	Final body weight Gm	Final blood pressure mm Hg	Carcass analysis						
			Water %	Fat % DT[1]	Na mEq/100 Gm FFDT[2]	K	Cl	N % FFDT[2] Gm	N % FFWT Gm
High Na controls	195	116	65 8	21 0	16 6	26 8	10 7	11 1	3 21
High Na + renal infarct	176	231*	70 0*	4 6	19 7*	21 0*	12 9*	10 0	2 91
Low Na controls	183	116	65 7	22 8	16 1	26 1	10 5	11 4	3 28
Low Na + renal infarct	185	207*	67 8*	15 5	16 0	25 5	9 8	10 6*	3 04*

[1] Dried tissue

Fat free dried tissue

[3] Fat free wet tissue

Significantly different from "normal" values. P = 0 05

Unpublished data

What relation these electrolyte changes bear to *hypertension* if any is not known. In order to determine whether these changes were an essential accompaniment of hypertension in general or were seen only in the hypertension induced by steroid a similar series of analyses was undertaken in rats made hypertensive by means of renal damage. Dr Bernt Hokfelt of the Karolinska Institutet in Stockholm was good enough to prepare for us a series of animals unilaterally nephrectomized and with an arterial infarct in the remaining kidney a technique first described by Dr Dorothy Loomis of the Long Island College of Medicine. To make the data more comparable to these described above the rats were placed on similar high and low sodium regimens.

Table VI shows the *data obtained in these animals along with control rats* observed at the same time. Body weights were comparable in all four groups. Blood pressure in the groups with renal damage was markedly elevated in all of the 11 animals in the high sodium group and less consistently so in the sodium restricted rats in which only four out of 10 became persistently hypertensive. The animals listed in Table VI in the low sodium group were the four most hypertensive members of their group. As compared to the normals on high salt the rats with renal damage showed significant increases in carcass water and sodium similar to the DCA injected animals. In addition *these renally damaged rats exhibited an elevation in chloride and a reduction* in potassium. Although sodium restriction did not alter the increased water content *it did prevent the electrolyte change*. It would seem that this renal hypertension was similar to DCA hypertension in that it was accentuated by a high sodium regimen and similar also in the character of the electrolyte changes which developed on this diet. It differed from DCA in that sodium restriction although it decreased the hypertensive action of the renal damage did not prevent it entirely. This however may be a quantitative rather than a qualitative difference and the over all similarity between the two is striking. On the contrary *cortisone hypertension is distinguished from the above* by its independence of sodium intake and by the fact that it may occur without *overt evidence of renal damage*

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STUDIES ON THE PATHOGENESIS OF THE HYPERTENSIVE VASCULAR DISEASE WHICH OCCURS IN RATS BEARING REGENERATING ADRENAL CORTICAL TISSUE*

FLOYD R. SKELTON, M.D., Ph.D. *Director of Research, the Urban Maes Research Foundation, and Associate Professor of Pathology Louisiana State University Medical School, New Orleans, La.* †

Whether or not some functional abnormality of the adrenal cortex plays a role in the pathogenesis of hypertension and hypertensive cardiovascular renal disease still remains one of the major problems confronting investigators in this field. The lack of unanimity of opinion on this question is well illustrated by statements from two well known investigators, one of whom(1) has said that "these glands probably play an important part in the pathogenesis of hypertension," while the other(2) has stated that "it is equally unjustifiable to conclude that the adrenal cortex . . . plays an important role in the pathogenesis of the disorder."

Much of the evidence supporting adrenal cortical participation in the genesis of hypertensive vascular disease has been derived from observations on the hypertensive and angiotoxic properties of various steroid (3, 4, 5), anterior pituitary substances(4, 6) and chronic exposure to cold(4, 7). On the basis of these studies, it was postulated(8) that under suitable conditions both hypertension and certain types of cardiovascular disease might be the result of an intense response of the anterior pituitary-adrenal cortical system to nonspecific stress. The original concept that these changes were simply the result of increased anterior pituitary secretion of adrenocorticotrophic hormone (ACTH) is tenable no longer since nonspecific stress, with the single exception of cold(7), and the administration of large doses of adrenocorticotrophic hormone have been notably unsuccessful in producing either hypertension or vascular lesions(4). Nevertheless, recent observations have generally upheld the hypothesis that this gland does play an important yet poorly understood part in their pathogenesis. For example, it has been shown that

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† This work was done while the author was Assistant Professor of Pathology, Medical College of Georgia, Augusta, Ga.

growth hormone administration to uninephrectomized salt treated rats results in elevated blood pressure and widespread vascular changes when the adrenals are intact(9) but not when these glands are absent(10) Furthermore it has been demonstrated that the most potent naturally occurring mineralocorticoid aldosterone is excreted in increased amounts by patients with essential hypertension(11 12) and produces moderate hypertension when administered to intact rats(13 14) Additional evidence implicating the adrenal cortex in the pathogenesis of hypertensive vascular disease is the observation that hypertension and necrotizing vascular lesions occur during regeneration of the adrenal cortex in uninephrectomized immature rats given 1 per cent sodium chloride to drink(15) Since the only difference between the rats which become hypertensive and the control animals which do not is the presence of the regenerating adrenal gland this new form of experimental hypertension has been termed adrenal regeneration hypertension (16)

This new technique for the production of experimental hypertensive vascular disease has afforded an excellent opportunity to study the interplay *between the endocrine electrolyte and metabolic factors which contribute to* the pathogenesis of both the hypertension and the vascular lesions For example recent investigations(17) have shown that regenerating adrenal cortical tissue is essential for the development of these changes since inhibition of regeneration by either hypophysectomy or the presence of the contralateral adrenal gland completely prevents them from taking place Furthermore reduction in renal mass by unilateral nephrectomy and provision of 1 per cent sodium chloride to drink are also necessary for the hypertension and vascular changes to occur(17) The development of this form of experimental hypertensive vascular disease is also influenced by age for it has been shown to occur in immature rather than in mature rats(18) Studies now in progress have demonstrated that it is inhibited by thyroidectomy(19)

The results of the present experiment contribute additional information on the role of regenerating adrenal cortical tissue not only in the genesis of this form of experimental hypertensive vascular disease but in its maintenance as well

EXPERIMENTAL PROCEDURE

Sixty female Sprague Dawley rats weighing 12 56 Gm were uninephrectomized and maintained on Purina Laboratory Chow and 1 per cent sodium chloride drinking solution *ad libitum* Ten rats received no further treatment and served as controls (Group I) The remaining 50 rats had the right adrenal completely removed and the left adrenal enucleated according to the method described by Ingle and Higgins(20) In this procedure the adrenal capsule is incised and the medulla and as much as possible of the cortex

extruded by the gentle application of pre sure with curved forceps thus leaving only a few cells of the zona glomerulosa attached to the capsule which remains *in situ*

Ten of the adrenal enucleated rats received no further treatment (Group III) At the end of six weeks an additional 10 rats were subjected to re enucleation of the regenerated adrenal by the same procedure as that originally employed (Group IV) and the remaining 30 had the regenerated gland completely removed Five of the re enucleated rats and five of the adrenalectomized animals died within 48 hours as a result of hemorrhage from either the re incised adrenal capsule or severed adrenal arteries Since these animals had severe hypertension and many had shown signs of cerebral lesions they have been considered together (Group II) with a view to obtaining information as to why some rats rapidly develop hypertension and vascular changes following adrenal enucleation while others do not Twenty two rats survived excision of the regenerated adrenal until the end of the experiment and of the e animals 19 exhibited con istent changes in blood pressure and saline consumption that differed considerably from the three other rats Thus the 19 rats have been considered together as Group V and the remaining three animals make up Group VI

Body weight and systolic blood pressure the latter by the microphonic manometer method of Friedman and Freed(21) were determined initially and weekly thereafter throughout the experiment The consumption of 1 per cent sodium chloride drinking fluid was measured daily and the average 24 hour intake per rat was calculated weekly for each group The organs from the animals which died after six weeks were removed weighed fresh on a Cramatic balance and fixed in Bouin s olution At the end of 12 weeks all rats were killed by decapitation and their organs handled similarly Paraffin ections were prepared from the ti sues to be studied and stained with hematoxylin and eosin

RESULTS

The average weekly body weight saline consumption and systolic blood pressure of Groups I II III and IV are shown in Figure 1 and of Groups I III V and VI in Figure 2 The organ weights are pre ented in Table I Before considering this data however it should be noted that one control rat died and that only five rats in Group III and four rats in Group IV urvived the 12 weeks of the experiment The animals which died in Group III all had hypertension and va cular lesions and their death was the result of cerebral infarction econdary to the e changes The deaths in Group IV were due to po toperative hemorrhage at ix weeks hence these rats became part of Group II

TABLE I

Influence of Adrenal Re enucleation and Adrenalectomy on the Organ Weights of Rats with Adrenal Regeneration Hypertension

Group	No of rats	Duration in weeks	Average organ weights in mg						
			Heart	Kidney	Brain	Liver	Adrenal (s)	Spleen	Thymus
I Control	9	12	908 ±26*	1588 ±63	1735 ±5	7515 ±320	65 6 ±2 6	583 ±27	256 ±13
II** Adrenal enucleation	10	6	1033 ±38	2007 ±94	1835 ±47	9369 ±480	46 2 ±2 5	831 ±61	214 ±10
III Adrenal enucleation	5	12	1118 ±68	2314 ±240	1773 ±30	10820 ±1400	52 7 ±3 1	736 ±31	260 ±26
IV Adrenal enucleation + Adrenal re enucleation Six weeks	4	12	1250 ±12	2132 ±160	1804 ±28	11040 ±215	19 6 ±5 6	710 ±19	176 ±26
V Adrenal enucleation + Adrenalectomy – six weeks Decline of hypertension	19	12	950 ±18	1732 ±50	1830 ±16	8276 ±210		719 ±13	199 ±26
VI Adrenal enucleation + Adrenalectomy – six weeks Persistent hypertension	3	12	1303 ±118	2302 ±290	1911 ±60	9611 ±400		805 ±48	169 ±44

Standard error of the mean.

* This group consists of rats which died within 48 hours following adrenalectomy or adrenal re-enucleation at six weeks.

The rats which died postoperatively at the sixth week of the experiment (Group II) showed significant growth retardation while the rats of the other groups grew at essentially the same rate (Figure 1). The animals of Group II also developed the highest blood pressure and drank significantly more 1 per cent sodium chloride solution than did the control. Although the rats of Group III slowly became hypertensive, their blood pressure was significantly lower than the systolic blood pressure of the rats in Group II at six weeks and never reached the level attained by the latter animal. It is also noteworthy that the saline consumption of the rats in Group III was significantly lower than in Group II at six weeks and indeed did not differ significantly from the intake of control rats (Group I) throughout the experiment. The saline intake and blood pressure values of the re enucleated rats (Group IV) were between those of Groups II and III at the sixth week and did not differ significantly from them. After adrenal re enucleation the saline intake and systolic blood pressure of these animal decreased slightly but not significantly for two weeks followed by a rise to levels above those which had existed before the regenerated *adrenal was re enucleated and higher than those which were* attained by the rats of Group III.

There were no significant differences between the growth curves of any of the groups shown in Figure 2. The blood pressure and saline consumption were virtually identical in Groups III and V for the first six weeks of the experiment but *following removal of the regenerated adrenal gland in the rat* of Group V the blood pressure decreased to a level approximately that of the controls (Group I) while the saline intake dropped below that of either Groups I or III. The changes observed in the identically treated animals of Group VI were considerably different. Both blood pressure and saline consumption of these rats decreased for two weeks after excision of the regenerated adrenal but increased thereafter to approximately the same levels which had existed before the operation and similar to those in Group III.

In Table I it can be seen that the hearts of all hypertensive animals (Groups II, III, IV and VI) were significantly hypertrophied as compared to controls (Group I). The absence of cardiac hypertrophy in rats in which the regenerated adrenal had been removed after six weeks and the blood pressure had returned to normal levels (Group V) contrasts markedly with the cardiac hypertrophy in similarly treated rats (Group VI) in which the hypertension persisted. The changes in renal weight essentially paralleled the changes in cardiac weight hypertrophy being present in all groups exhibiting hypertension and absent in rats with normal blood pressures. The weight of the brain was slightly increased in all groups as compared to control values but the increase was significant only in Group II, IV, V and VI. The liver was significantly enlarged in the rat of Group II, III, IV and VI while no enlargement was present in the nonhypertensive rats of Group V.

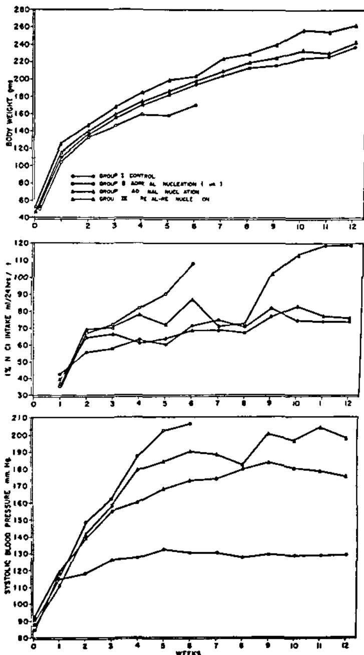


FIGURE 1 Influence of saline consumption and adrenal re-enucleation on the development of "adrenal regeneration hypertension"

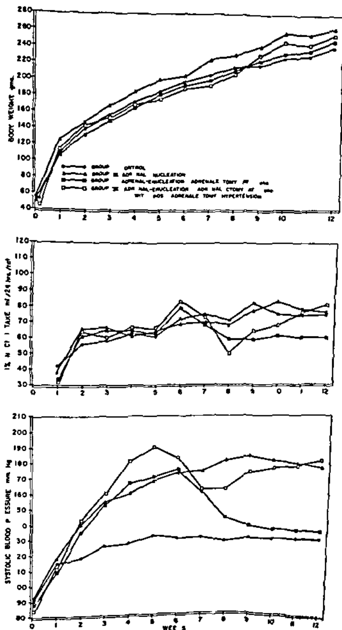


FIGURE 2 Influ nce of ad n le to y n the m nte ance of "a lrenal reg ration hyperten n"

TABLE II

Summary of Morphological Observations

Group	Kidney	Heart	Brain	Adrenal (s)	Pancreas and mesentery
I	Slight degeneration of glomerular capillaries focal tubular [illegible] dilatation No vascular lesions	No lesions	No lesions	No lesions	No lesions
II	Sclerosis and fibrinoid neurosis of glomeruli and arteriol tubular [illegible] dilatation and hyaline casts Severity of lesions 2 4	Arteriolar sclerosis and fibrinoid necrosis perivascular fibroblastic proliferation Severity of lesions 2 1	Edema focal hemorrhage arteriolar sclerosis and fibrinoid degeneration and cystic infarcts Severity of lesions 2 7	Sclerosis and fibrinoid necrosis of capsular arterioles with focal necrosis of cortex Severity of lesions 2 5	Periarteritis nodosa and sclerosis and fibrinoid necrosis of arterioles Severity of lesions 2 1
III	Like Group II Severity of lesion 1 8	Like Group II Severity of lesions 0 9	Like Group II Severity of lesions 0 6	Like Group II Severity of lesions 1 0	Like Group II Severity of lesions 0 4
IV	Like Group II Severity of lesion [illegible]	Like Group II Severity of lesion 1 4	Like Group II Severity of lesions 1 0	Like Group II Severity of lesions 2 0	Like Group II Severity of lesions 1 7
V	Hyperplastic arteriolar sclerosis present in 12 out of 19 rats	No lesions	No lesions	*Hyperplastic* sclerosis of capsular arterioles in 4 out of 19 rats *	*No lesions*
VI	Like Group II Severity of lesions 2.5	Like Group II Severity of lesions 0 8	Like Group II Severity of lesions 1 0	Like Group II Lesions in 2 out of 3 rats Severity 1 0*	Like Group II Severity of lesions 0 5

Adrenals removed at six weeks.

The weight of the regenerated adrenal gland in Groups II III and IV was significantly less than the weight of both adrenals in Group I Furthermore no significant difference existed between the weight of the regenerated adrenal at six and 12 weeks (compare Groups II and III) and no greater adrenal regeneration was produced by re enucleation (compare Groups III and IV) The spleen was enlarged in all experimental groups The weight of the thymus in the rats of Groups II and III was not significantly different from the thymus weight in control rats (Group I) but in the re enucleated animals (Group IV) this gland was significantly smaller On the other hand the thymus was significantly enlarged in both groups in which the regenerated adrenal had been removed (Groups V and VI) despite the difference in blood pressure in these rats

Morphological Studies Since a detailed presentation of the morphological changes which develop in rats with this type of experimental hypertension has been published elsewhere(16) they will not be described here other than in the brief outline contained in Table II In general the lesions are virtually the same as those which accompany experimental hypertension produced



FIGURE 3 Kidney of rat with persistent hypertension six weeks after complete removal of the regenerated adrenal gland (Group VI) Glomerulus shows fibrinoid necrosis and afferent arteriole shows hyperplastic arteriolosclerosis with marked narrowing of the lumen Hematoxylin and eosin × 100

in this species by a wide variety of procedures and except for periarteritis nodosa they closely resemble the changes of malignant hypertension in the human being The severity of the lesions has been evaluated grossly and microscopically on a scale of 0 to +++ and the figures for each group shown in Table II represent the proportion of the possible maximum

The most severe and widespread lesions occurred in the rats which died after six weeks (Group II) The hemorrhage causing the death of these animals probably was the result of the severe vascular sclerosis and fibrinoid necrosis of the adrenal arterioles which prevented these vessels from contracting after being severed The lesions in the rats which underwent adrenal re enucleation (Group IV) were more severe than in the adrenal enucleated rats of Group III but less severe than in the rats of Group II Except for slight renal arteriolar hyperplasia no lesions were observed in the animals of Group V On the other hand the similarly treated animals of Group VI showed changes identical with those observed in the other hypertensive rats Representative lesions from one of the animals in this group are illustrated in Figures 3 6

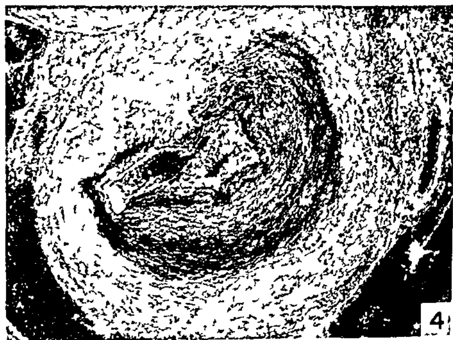


FIGURE 4 Periarteritis nodosa of pancreatic artery from rat with persistent hypertension six weeks after complete removal of the regenerated adrenal gland (Group VI) Note aneurysmal dilatation largely filled with organizing thrombus Hematoxylin and eosin × 68

FIGURE 5 Coron ry ve el of rat with persi tent hyperten ton x weeks fter c n plete *emoval of the reg n rated adren l gland (Group VI)* Not *early fib* noi *l* d g nerat on of the med a and the l rge f amy c ll in the i tima of tl ves l Hen atoxylin an l eo in × 3 0

FIGURE 6 Sect on of b in *from r t with p rsist nt hyperten on s* weeks co plet ren oval of the eg n rat l a lren l gl nd (Group VI) sh wing hyperr a t ri lo cler sis and unde lyi g old cysti infarct. H m toxyl n an l eo in ×

DISCUSSION

The present observations substantiate the important role of regenerating adrenal cortical tissue in the pathogenesis of the hypertension and vascular lesions which develop in rats following adrenal enucleation. Thus re-enucleation of the regenerated gland in rats already hypertensive from adrenal enucleation six weeks before produced only a slight and transient decline in blood pressure which subsequently rose above the pre-existing level as the cortex again regenerated. Furthermore complete removal of the regenerated adrenal at six weeks in similarly hypertensive rats produced a fall in blood pressure to normotensive levels in 19 out of 22 animals. On the other hand three such adrenalectomized rats remained hypertensive. While these results should be considered preliminary because of the small number of rats which remained hypertensive, they nevertheless suggest that adrenal regeneration hypertension is initially dependent upon the presence of regenerating adrenal cortical tissue but that it can become independent of adrenal cortical function. The persistence of hypertension after removal of the regenerated adrenal cortex in some rats is reminiscent of the hypertension ("meta-corticoid hypertension") which remains in rats after cessation of prolonged desoxycorticosterone acetate administration (22, 23, 24). The mechanism responsible for the maintenance of "meta-corticoid hypertension" or that hypertension which persisted after removal of the regenerated adrenal in three rats in the present experiment is not known. However, renal arteriolosclerosis is especially prominent (24) in animals with "meta-corticoid hypertension" and bilateral nephrectomy in such animals is followed by a reduction in blood pressure toward or to normal limits (24, 25). Hence the presence of severe renal lesions in the three rats with persistent hypertension and their virtual absence in similarly treated animals whose blood pressure returned to normal following removal of the regenerated adrenal suggests that some renal mechanism may be involved in the maintenance of the post-adrenalectomy hypertension in the former rats.

The 10 rats that died within 48 hours after the second operation (Group II) point up the etiological significance of sodium chloride in the genesis of adrenal regeneration hypertension and vascular disease. These rats developed the highest blood pressure and most severe vascular lesions and also drank the most saline solution. On the other hand the rats of Group III became hypertensive more slowly and generally showed less severe vascular changes than the rats of Group II; they never drank significantly more saline than control animals. In short, it has now been demonstrated that with only water to drink, adrenal regeneration hypertension does not develop (17); with a saline consumption no greater than uninephrectomized controls it does

develop while with a consumption of saline greater than the controls the greatest hypertension and mo t severe va cular lesions have occurred

A further correlation between blood pressure and aline intake was ob erved in their trikingly parallel changes following re enucleation and excision of the regenerated adrenal Whether the changes ob erved in the sodium chloride consumption under the e conditions reflect altered adrenal or renal function is not known However the fact that after removal of the regenerated adrenal the hyperten ive rats with renal lesions drank more aline than the normoten ive rats without renal lesions uggests that under these conditions at lea t the polydip ia was on a renal rather than an adrenal basis

As tated previou ly (16) the mechanism by which regenerating adrenal cortical tissue participates in the genesis of adrenal regeneration hypertension remains unknown Nevertheless it has been con idered of significance that hypertension and cardiovascular renal le ions result from conditions in which altered adrenocortical function presumably exi ts and in which non specific tress appears to play no part (15) It was originally suggested that the regenerating adrenal cortex might be ecreting increased quantities of gluco corticoid mineralo corticoid or both in respon e to the increased anterior pituitary secretion of ACTH induced by the sudden fall in circulating blood corticoids econdary to the adrenal enucleation Of the e suggestions perhaps the most rea onable i that an imbalance exi ts between the production of gluco corticoid and mineralo corticoid as a result of the enucleation procedure This would be quite compatible with the fact that enucleation removes those zones of the cortex which are felt to be associated with gluco corticoid secretion but leaves at least part of the zona glomerulosa which is considered the site of mineralo corticoid secretion and from which the adrenal cortex largely regenerates The sugge ted imbalance might be expected therefore to be in favor of mineralo corticoid secretion This possibility remains to be tested under *in vivo* conditions similar to those of the pre ent experiment although *in vitro* studies (26) have hown that the principal teroid ecreted by the zona glomerulosa of enucleated rat adrenal glands is aldo terone

SUMMARY

Severe hypertension and vascular lesions are produced in rats as the cortex regenerates following adrenal enucleation Previous experiments are referred to and discus ed The e demon trate that thi new type of experimental hypertensive vascular disea e develops in young growing female rats that have the renal mas reduced by uninephrectomy and are given 1 per cent sodium chloride solution to drink Although it is not necessary for rats bearing regenerating adrenal cortical ti sue to drink greater quantities of aline than control rats in order to develop hypertension and vascular lesions it has been

shown that excess sodium chloride must be available since replacement of the saline drinking solution with tap water prevents the hypertension and vascular changes The present experiment further implicates sodium chloride in the pathogenesis of adrenal regeneration hypertension and vascular disease since rats which developed the most fulminant hypertension and vascular lesions also consumed the greatest quantities of saline

The present group of related experiments also shows the effects of (a) a second enucleation and (b) removal of the regenerated adrenal six weeks after the first enucleation when hypertension was present Re enucleation of the regenerated adrenal in four rats produced a transient decrease in blood pressure and saline consumption followed by a rise to above the pre existing hypertensive levels Complete removal of the regenerated adrenal caused a fall in blood pressure from hypertensive to normotensive levels accompanied by a drop in saline consumption to below normal limits in 19 rats while three similarly treated hypertensive animals showed only a transitory fall in blood pressure and saline intake which returned to preoperative levels The presence of moderate to severe arteriolar lesions especially of the kidney in the three rats which remained hypertensive and the finding of only slight thickening of the renal arterioles in 12 of the 19 rats which became normotensive suggest that the hypertension in the former animals had become independent of the regenerated adrenal and was maintained by some renal mechanism

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STUDIES ON THE REVASCULARIZED KIDNEY*

YALE J KATZ, M D, Ph D, *Associate Professor of Medicine*
Department of Medicine, University of Southern California
Los Angeles, Calif

The idea of increasing the kidney s blood supply in order to improve its function or lower blood pressure is not a new one According to Siter(1) the first attempt at renal revascularization was made by Tuffier of Paris in 1890 In 1901 Edebohls (2) of New York decapsulated the kidneys of a number of patients with chronic nephritis He believed that the beneficial effects of this procedure resulted from the growth of new blood vessels into the kidney from the surrounding structures A few others(1) shared this enthusiasm for renal revascularization but in general little attention was paid to the subject until Paunz(3 4) in Germany and MacNider and Donnelly(5) in the United States grafted omentum into the incised cortex of the kidney Paunz occluded the renal artery of several dogs after this procedure

Following Goldblatt s(6) observation that some of his hypertensive dogs became normotensive if they developed spontaneously an accessory renal blood supply interest in the deliberate revascularization of the kidney was renewed In addition to omentum(7 8) muscle(8 9 10) and spleen(11 12) came into use as blood vessel donor tissues There were a few observations(11 12) of amelioration of hypertension in dogs following some of these procedures but results in human hypertension and renal insufficiency were disappointing(9 13)

It appears to be not generally appreciated that for the satisfactory revascularization of any organ the blood vessel donor must be endowed with a rich blood supply and in the case of renal revascularization the blood reaching the kidney must be at a sufficient head of pressure to support glomerular function

Baronofsky Sprafka and Noble(14) used a loop of intestine for the revascularization of the heart Because of its rich blood supply and the relatively small drop in arterial blood pressure between the aorta and the intestine a segment of intestine seemed ideal as a donor of blood vessel for the kidney

These studies for the most part have been described elsewhere(15 16) They show that vascular communication between the intestine and the kidney can be developed and that kidney function can be supported by such a blood supply

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FIGURE 3   l ar um sulf te injecte l into branch of mesenteric n attacl d to ki lney

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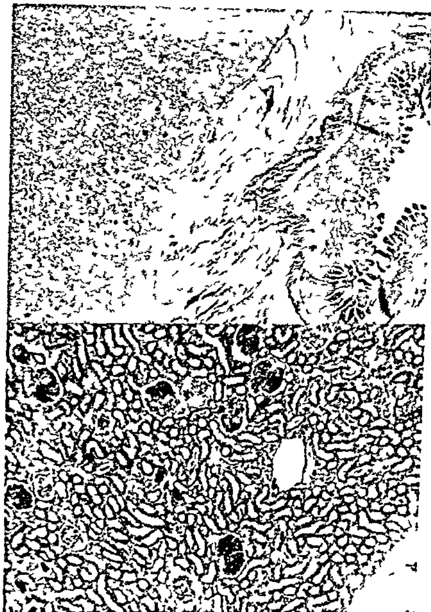


FIGURE [illegible]. Showing effect of injecting a dilute suspension of India ink into the nutrient vessel of a segment of intestine fixed to the kidney. Above can be seen the intestine and the kidney on cross section under low power magnification. Below is a high-power magnification of a section of kidney showing India ink in the glomeruli.



FIGURE 3 Radiograph showing barium sulfate injected into branch of mesenteric artery of segment of small intestine attached to kidney

TABLE II

Acute Renal Artery Occlusion and Indigo Carmine Excretion

Dog	Type of renal revascularization	Renal artery occlusion	Indigo carmine injection	Dye appearance in urine
XXI	L Nephrocolonopexy–2 months	5 56 p m	2 0 ml	6 10 p m
	R Nephroduodenopexy–2 weeks		6 03 p m	
XXII	R Nephrojejunopexy–3 months	6 03 p m	1 0 ml	6 10 p m
	L Nephro ileopexy–6 weeks		6 05 p m	
XXIII	Bilateral nephrojejunopexy–1 month	4 52 p m	1 0 ml 4 53 p m	5 04 p m
XXIV	R Nephro ileopexy–4 months	1 34 p m	1 0 ml	1 59 p m
	L Nephrojejunopexy 10 weeks		1 50 p m	
XXV	L Nephrocolonopexy	3 11 p m	2 0 ml 3 12 p m	None by 3 15 p m
			2 0 ml 3 25 p m	Released renal artery clamps 3 50 p m

after occlusion of the renal arteries had their renal arteries transected in most instances after total occlusion of the renal arteries (approximately three to six weeks) Table III lists the animals so studied and the effect of renal

TABLE III

Animal Survival and Plasma Nitrogen After Renal Artery Transection

Dog	Plasma urea nitrogen			Survival
	Before transection	After transection		
	(Average)	(Maximum)	(Average)	
I	13	29	22	Sacrificed after 2½ months
II	19	27	17	17 months still living
III	18	32	11	22 months still living
IV	21	25	12	18 months still living
V	14	25	12	17½ months still living
VI	12	27	23	6 months dead
Av	16 7	28 9	16 2	

artery transection on blood urea nitrogen and animal survival and includes follow up studies on dogs previously reported(10) Table IV lists the renal function in dogs whose renal arteries have been transected or occluded

TABLE IV

Effect of Interruption of Renal Artery on Renal Function in Revascularized Kidneys

Dog	Before transection			After transection		
	Renal blood flow (ml/min)	Glomerular filtration rate (ml/min)	Filtration fraction (per cent)	Renal blood flow (ml/min)	Glomerular filtration rate (ml/min)	Filtration fraction (per cent)
I	200	45	41	174	43	38
V	250	56	58	299	57	37
VI	227	54	41	171	41	42
*VII	243	49	35	122	25	32

*Renal artery totally occluded but not transected

DISCUSSION

It appears from these studies that the kidney of the dog can do well with a blood supply which is entirely peripheral

Cook and Pearson (17) report a case of a 28 year old man with bilaterally thrombosed renal arteries The source of the kidneys blood supply was from dilated capsular vessels Prior to death the urea clearance was 75 per cent of normal

One might question the wisdom of introducing additional blood in the kidney of patients suffering from renal arteriosclerosis or chronic glomerulonephritis when such kidneys do not lack for blood at their portal Yet once the blood enters the kidney it is up against an increased resistance to its flow out to the cortex because of scarring and contraction Therefore it is possible that bringing additional blood to the nephrons by a peripheral type of accessory blood supply would improve the kidney's function

One possible method of achieving this purpose has been described There is nothing to suggest that the procedure harms the kidney and studies to be reported later suggest that under some circumstances renal blood flow in dogs is increased

Acknowledgement

This study represents the combined efforts of a number of individuals The author is grateful to Dr Ivan D Baronofsky in whose laboratory at the Ancker Hospital St Paul Minnesota the investigations were initiated and to Dr Donald Hannon John Alden Clark Turback and Joseph Sprafka of the University of Minnesota who gave generously of their time and surgical skill

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THE ELECTRON MICROSCOPY OF THE KIDNEY*

Summary of a Review Presented to the High Blood Pressure Research Council Meeting, 1956

DANIEL C PEASE, PH D , *Professor of Anatomy, Department of Anatomy School of Medicine University of California, Los Angeles, Calif*

INTRODUCTION AND METHOD

Our present understanding of the complex structure of the kidney has been developed during three historical periods When sectioning techniques suitable for electron microscopy were first being devised the kidney was studied in Bethesda in Paris and in my laboratory in Los Angeles By current standard the work was crude As methods improved Dr B Vincent Hall at the University of Illinois (1) devoted his efforts to a study of the glomerulus and added greatly to our knowledge of this important part of the kidney In the third historical phase currently acceptable methods were employed to obtain high resolution electron micrographs of adequately preserved material The Karolinska Institutet laboratory in Sweden (2 3) and my laboratory (4 5) have been particularly concerned with this work

Osmic acid carefully buffered is the fixative of choice for almost all electron microscopy that deals with tissue sections In our laboratory the most satisfactory results were obtained when an ice cold solution of fixative was dripped on the exposed surfaces of kidneys of living animal after removal of the capsule The fixative penetrates establishing a concentration gradient Just below the surface is a zone particularly well preserved Obviously this technique is limited to the cortex when the medulla is to be studied excision techniques are required The preservation in that case is not as good Human material of course cannot be handled in this manner and this imposes a definite limitation on the work that may subsequently be done

Glomerular Capillaries Electron microscopy has demonstrated that glomerular capillaries have a surprisingly complex and unexpected structure Epithelial cells are suspended in the urinary space and make contact with the capillary wall only by myriads of tiny terminal processes or feet The terminal processes from different cells or from different arms of the same cell form systems which interdigitate with one another everywhere over the surface of

* Supported by grant from the United States Public Health Service

the capillaries There are slots between adjacent feet so that this layer does not constitute a barrier to diffusion processes On the endothelial side of the capillary perikarya abruptly transform into excessively thin sheets of cytoplasm that extend long distances constituting the great proportion of the capillary lining The attenuated endothelium is perforated by large numbers of pores These usually measure a little over one tenth of a micron in diameter and are closely spaced one beside another They are seen particularly well in tangential sections of the capillary wall Obviously such a fenestrated endothelium cannot be regarded as an important diffusion barrier

The only continuous barrier between the blood stream and the urinary spaces is the basement membrane Three extracellular layers constitute this structure *In the center is a layer of dense material that is probably the* principal structural component On either side of this dense lamina are zones of low density On the outer surface the epithelial feet penetrate slightly into one of these zones and on the other side the endothelial sheet rests upon a similar zone One might wish to call such layers cement layers and they apparently serve to bind the cellular components to the structural part of the basement membrane In tangential section these layers of the basement membrane can be viewed in the horizontal plane In the author s experience they are homogeneous in character even when viewed with great resolution although other investigators have claimed that they observed suggestions of fibrous components It seems necessary to regard this basement membrane as the only important filtration barrier between blood and urinary space but it does not seem possible that it could provide any regulation of the urine output Possibly regulation might be provided by changes in the size of the endothelial pores or by swelling of the epithelial feet so that the gaps between *them would be more or less obliterated We have however no physiological* evidence to support such hypotheses

Peritubular Vascular Bed The peritubular capillaries also have a notably thin endothelium In suitable preparations it can be seen that this attenuated sheet is also perforated by a system of pores The pores are markedly smaller than in the case of the glomerular capillaries being approximately 6/100 μ in diameter Also they are spaced further apart In the medulla the system *is not as well developed as in the cortex and there one find only patches of* fenestrated endothelium Once again we are forced to conclude that the fenestrated endothelial sheet represents a *specialization facilitating diffusion* processes rather than constituting a diffusion barrier

The endothelium of the peritubular capillaries rests upon a basement membrane The dense structural portion of this membrane is extremely thin and is in striking contrast to the thick structural membrane of adjacent tubules A thin cement layer lies between the dense portion of the basement membrane and the endothelium

The Tubules Basal Infolding Probably the most important contribution of electron microscopy to tubular morphology is the discovery of the extraordinary infolding or intussusception of the plasma membrane demonstrable in the basal regions of tubular cells By this means the basal surface area presumably is vastly increased The infolding is most elaborately developed in the distal tubule where the osmiophilic line representing the plasma membrane folds deeply into the cytoplasm and then reflects back to reach the surface once more This repeats over and over again to form deep scallops These folds anastomose freely with one another laterally so that in horizontal section one sees a veritable honeycomb of membranes The Swedish investigators (3) who first saw this well preserved emphasized that this system of double membranes divided the basal cytoplasm into open ended compartments which tended to contain mitochondria correspondingly oriented We have physiological evidence that these membranes are in fact the effective physiological surface of the cells and thus truly the plasma membrane of the basal end

The proximal tubule shows a considerable development of this system of infolded basal membranes which are also associated with the mitochondrial zone The collecting tubule shows this system as well although the folds do not penetrate deeply into the cell Nonetheless they ramify greatly in a lateral direction In the collecting tubule the mitochondria are in a totally separate zone so that relation between these two systems may be entirely fortuitous

In our laboratory we wondered whether this specialization would be found in other tissues noted for their water transport mechanism We have examined the choroid plexus of the brain ventricles and the epithelia of the ciliary body of the eye and of salivary gland In all of these cases we have found analogous infoldings of the basal surface of the cells Folding of this sort is not known to occur in other epithelia which are not involved in water transport There would seem to be legitimate reason for postulating that there is cause and effect relationship between the infolded basal surface and water transport It is particularly interesting that the collecting duct of the kidney shows something of this system and therefore presumably would have some physiological activity Furthermore it is tempting to think of the infolded membranes as being dynamically active and a degree of regulation might be accomplished by alteration in the total surface area Again we must emphasize that this is a hypothesis unsupported by physiological evidence

Proximal Tubule Apical Region The brush border of the proximal tubule may be demonstrated very beautifully by electron microscopy There is no doubt but that it represents a simple extension of the apical cytoplasm

Here and there between bru h proce e tiny ducts may be een penetrat ing into the apical cytopla m These ometimes open into vacules of con siderable size This sy tem can be identified as a *specialization of the o called* endopla mic reticulum This may be defined as a sy tem of mem branes delimiting cisterns The membranes are peculiar in that they have an affinity for RNA granules Inve tigators at the Rockefeller Institute have been tudying this y tem in many different cell types In certain in tance the sy tem communicates with cell surface and is ometimes involved in pick ing up material in other in tance it is known to be involved in the elimina tion of material Thus in relation to the proximal tubule we cannot be cer tain whether we are ob erving an acce sory excretory sy tem or whether it is a means for picking up material from the tubular lumens and thus in corporating them within the cell

Both Rhodin (2) and Pea e (5) have reported un uccessful efforts to cor relate changes in the proximal tubule with different physiological load upon the kidney Small differences in the way kidney amples are prepared produce large variations in the observed morphology of the proximal cell These terminal change as fixative is applied probably overwhelm any original patterns The terminal changes are such as to indicate that the apical end of these cell is very labile and can quickly pick up or lo e water

Thin Segment Electron micrographs of the thin egment portion of the nephron reveal one interesting pecialization The cell have folded edges so that neighboring cells interdigitate with one another in a remarkably com plex manner It would seem that these interdigitations greatly increa e the available cro s ection area of intercellular pace that might be a route for ionic exchange as Chambers sugge ted many years ago in regard to capillary endothelium

Mitochondria Rhodin (2) has followed by electron micro copy the mito chondrial changes een during the resorption of albumin He reports an appar ent *fu ion of mitochondria a an early respon e to protein resorption followed* by a lo s of their characteri tic internal tructure as they combine with the re sorbed protein This re ults in the production of very large granules without internal structure other than a fine granulation Eventually the large granules eem to reconvert back into typical mitochondria Both the proximal and di tal tubules are unu ually rich in mitochondria El ewhere mitochondria are of only moderate size and abundance

RNA Granules It is now po ible to characterize RNA granules with the electron micro cope It may be noted that the cytoplasm of the proximal tubule cell is notably rich in the e organelles *This gives it the pronounced* basophilia well known to the light micro copi t Although some of these

granules are found in other portions of the nephron they are not notably concentrated elsewhere

CONCLUDING REMARKS

An effort has been made to discuss the newly discovered cytological pecial izations of the kidney In general these morphological details could not have been revealed by conventional micro copy they demanded the resolution made possible by electron microscopy It is surprising how complex this organ now appears to be yet no doubt we will find in this complexity new ways in which the kidney can perform its precise regulatory function Un doubtedly the future will see this new morphology coupled with physiological and pathological knowledge This will constitute the fourth and most impor tant epoch in our tudy of the kidney by electron microscopy

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SOME FACTORS INFLUENCING THE RENAL EXCRETION OF SODIUM AND WATER

H. L. White, M.D., *Professor of Physiology, Department of Physiology, Washington University School of Medicine St. Louis, Mo.*

The rates of excretion of sodium and water represent the differences between the rates at which they are filtered and at which they are reabsorbed by the tubules. Our present task is to consider the factors which can effect, in normal or abnormal state, alterations in these differences. If increase in reabsorption equals increase in filtered load, there is no change in excretion. Retention means merely that output has fallen below intake, and this is because tubular reabsorption is excessive for the current filtered load. We do not know why this occurs in, for example, congestive failure, but we may consider briefly some of the possibilities.

FILTRATION RATE

It is well established that in the case of normal kidneys an acutely produced small percentage fall in glomerular filtration rate (GFR) as, for instance, by mechanical compression of the renal artery, by a fall in systemic arterial pressure or by postural changes, brings about large percentage acute falls in rate of sodium and water excretion. That is, reabsorption is temporarily excessive for the reduced filtered load. If, however, the fall in GFR is maintained, output rises to normal, i.e., reabsorption falls until output again equals intake. As examples, the chronic Goldblatt dog does not show edema nor does the hypophysectomized dog with chronically reduced GFR. We may postulate that the transitory retention produced by the temporary persistence of reabsorption which is high relative to the reduced filtered load eventually signals the tubules, directly or indirectly, that a surplus exists and reabsorption falls until output again equals intake. We can only speculate as to whether this adaptation in tubular behavior is brought about (a) by a slight and not easily detectable increase in plasma sodium concentration which signals the tubules either directly or via a reduction in output of salt-retaining adrenal steroids, (b) by volume receptors which bring about alteration in tubular activity in some unknown way (as by reflex suppression of ADH or aldosterone secretion?), or (c) by some other unidentified process or combination of processes.

We do have experimental evidence that if such temporary retention is prevented the excessive tubular activity relative to the reduced filtered load persists and the sodium and water outputs of the affected kidney remain low as long as its GFR is low. This is shown as follows (1) With trained unanesthetized dogs previously operated upon to permit separate urine collections from the two kidneys the outputs of sodium and water on the two sides are essentially equal as are GFR and renal plasma flow (RPF). With chronic constriction of one renal artery sufficient to produce permanent *small* percentage falls in GFR and RPF in that kidney the outputs of sodium and water by that kidney show permanent *large* percentage falls persisting as long as the constriction is maintained. This is presumably because retention has been limited by the undisturbed companion kidney in which output of sodium and water rises until the combined outputs of the two kidneys equal the normal for the two kidneys, i.e. until output equals intake. If hypertonic saline is given intravenously both kidneys show a great and equal percentage rise in sodium output; the output of the kidney with the constricted artery remains the same small fraction of that of its companion. If the constricting ligature is removed the output of the previously constricted kidney rises and that of its companion falls until they are again equal, their sum continuing to equal the intake. This cycle may be repeated.

A numerical example with no pretense of quantitative accuracy may illustrate this viewpoint. Assume a steady state with GFR of 100 ml/min, sodium concentration in filtrate of 14 mEq/100 ml and sodium excretion of 0.14 mEq/min. With each kidney excreting 0.07 the tubules of each kidney are set to reabsorb 6.93 mEq/min, which they will continue to do until something happens to change their behavior. Constriction of one renal artery drops the GFR on that side to 45 ml/min with no immediate change on the other side. With 6.3 mEq/min now filtered on the constricted side and the tubules (since there has been no change in plasma composition) still set to reabsorb 6.93, none would be excreted if reabsorption continued at its former rate. Since removal of the last traces of sodium is difficult reabsorption falls a little short of being complete, with 6.293 reabsorbed and 0.007 excreted. With GFR 90 per cent of its preconstriction value, sodium excretion on that side is only 10 per cent. Because of diminished total excretion sodium retention develops provided intake is maintained. The surplus of sodium signals the tubules to cut down on reabsorption. As this signal develops the output from the unconstricted side rises until balance is re-established. This signal also operates on the constricted side but produces only a small absolute rise in excretion from that side since the reabsorptive set can be reduced considerably and still exceed the amount filtered.

With moderate constriction the sodium and water outputs from the constricted side may be only 10 per cent of those of the companion while GFR and RPF are 80 to 90 per cent If now the companion kidney is removed the remaining kidney's artery still being constricted its sodium and water outputs quickly rise from say 10 per cent of the intake to equal the intake At the same time its GFR and RPF rise to a level somewhat higher than that of one normal kidney but considerably lower than that of the two normal kidneys Here again we see normal output of sodium and water with chronically reduced total GFR We thus see that bilateral reduction in the GFR produces only temporary reduction in the output of sodium and water and unilateral reduction in the GFR produces persistent reduction in the output on that side so long as the companion kidney is present But after removal of the companion the output of the remaining constricted kidney rises to normal even though its GFR (in spite of a rise above that seen while the companion was present) is still below the normal for the animal

We can go further and apply a constriction so severe that the kidney becomes completely anuric although blood flow persists as shown by x ray examination (nephrogram) after intravenous Urokon injection(2) If now the companion kidney is removed urine flow from the severely constricted kidney is re-established and the animal survives without sodium or water retention With the GFR at zero or very low in the anuric or near anuric constricted kidney before removal of its companion the GFR rises to about that normal for one kidney but fails to rise as high as it does after unilateral nephrectomy with the remaining kidney not constricted These dogs showed arterial hypertension in the postconstriction prenephrectomy stage (rise from control mean of 112 to mean of 150 mm Hg) with no further rise after removal of the unconstricted kidney Thus the resumption of filtration and urine flow is not explained by a further rise in perfusing pressure but must be due to a fall *in preglomerular* renal vascular resistance Striking elevation of plasma urea after removal of the companion kidney was only temporary We thus see that when one renal artery is constricted so severely that urine flow from that kidney completely or almost completely ceases urine flow from that kidney adequate to maintain sodium and water balance and an almost normal plasma urea level is reestablished after removal of the companion kidney without further increase in arterial pressure This speaks again st the view that hypertension is a compensatory process to ensure adequate kidney perfusion The mechanism by which vascular resistance in a remaining constricted kidney falls after removal of its unconstricted companion is presumably the same as after unilateral nephrectomy where the remaining kidney is not constricted the responsible factors are unknown

These experiments speak against the view that sodium and water retention in congestive failure can be referred to a lowered GFR. With bilateral chronic reduction in GFR normal tubules respond by diminishing their reabsorption of sodium and water until output again equals intake. The ability of the tubules to alter their reabsorptive activity toward sodium and water so that output equals intake (on a day to day or week to week although not necessarily on a minute to minute or hour to hour basis) may indeed be taken as the definition of normal tubular behavior so far as sodium and water are concerned. The retention of sodium and water in congestive failure is then by definition the result of an abnormal behavior of the tubules, i.e. reabsorption from the filtered load of such an amount that the quantity escaping reabsorption is less than the intake.

This does not mean that we must consider congestive failure to be due to primary disease of the tubules. The tubules may be quite normal but responding with undesirably high reabsorptive activity to exaggerated factors stimulating reabsorption. Before we can discuss this matter with any degree of satisfaction we must know more about these factors than is now known. Four of these may be mentioned here: changes in plasma sodium level, in GFR and in extracellular fluid volume and finally hormonal influences.

An important recent observation demonstrating increased tubular reabsorption of sodium in congestive failure is reported by Barger et al (3). In a dog with two bladder pouches permitting urine collection from the separate kidneys, a small catheter is chronically implanted in a renal artery. Infusion of small amounts of a hypertonic saline solution into one artery of a normal dog produces a fivefold to tenfold increase in sodium excretion by the injected kidney without a detectable change in the GFR or the RPF and with no significant change in excretion on the other side. This means diminished tubular reabsorption not dependent on GFR changes or systemic hormonal influence. Presumably the tubular cells here change their behavior in direct response to the locally increased saltiness of the plasma and filtrate. It may be that this normal acute response is simply an unchanged rate of reabsorption in mM per unit time in the presence of an increased filtered load of sodium; in any event the end result is the rejection and excretion of more sodium per unit time in response to the local increase in concentration. The same experiment in a dog in congestive failure however shows no increase in sodium excretion on the injected side, i.e. the tubules reabsorb all the excess salt presented to them in the filtrate. *These failure dogs showed a normal* GFR but a low RPF. This convincing proof of increased tubular reabsorptive activity for sodium in failure still leaves the mechanism unexplained. Less expected is the authors' finding that injection of cardiac glycosides into one renal artery in amounts insufficient to produce systemic effects also increases sodium excretion from that side in dogs in failure.

FACTORS INFLUENCING TUBULAR REABSORPTION OF SODIUM AND WATER

Plasma Na Level If the plasma sodium level is raised within physiological limits by oral ingestion or intravenous injection of hypertonic saline tubular reabsorption of sodium may diminish i e excretion is increased even though the GFR may be unchanged Even though the GFR and the absolute amount of sodium reabsorbed per unit time may be increased when plasma level is raised one can at least say that a smaller percentage of the filtered sodium is reabsorbed This tubular response is more prompt in the dog than in man It may be due to a direct sensing by the tubules of the increased saltiness of the plasma or indirectly to a diminished output of salt retaining steroid or to both Evidence for the second mechanism is seen in the histochemical and cytological changes in the zona glomerulosa of the adrenal cortex following increased salt intake Conversely if the plasma sodium level is lowered even slightly as by salt deprivation or by dilution with water drinking tubular reabsorption of sodium becomes more nearly complete i e output falls even without a discernible fall in filtered load There is no evidence however that the increased avidity of the tubules for sodium in congestive failure is due to a lowered plasma sodium level The above cited observations of Barger et al indeed show that such increased avidity persists even when the plasma sodium level is locally increased This is in keeping with the previous finding that the failure patient fails to excrete a salt load in the normal fashion

Changes in GFR Some aspects of the effects of changes in GFR on tubular reabsorption of sodium and water have been discussed above but it may be appropriate to consider here the question of Tm^{d}_{Na} The suggestion(4) that distal reabsorption of sodium is limited by a maximal rate is recognized to be an oversimplification If during abundant sodium excretion (distal sodium reabsorptive mechanism presumably saturated) GFR is reduced it is true that sodium excretion will show a large fall perhaps almost to zero If Tm^{d}_{Na} were constant sodium excretion should remain low until the distally presented load of sodium rises (through a rise in plasma level with continued intake) to the control value But sodium excretion will rise long before the plasma level shows such a rise indicating that T^{d}_{Na} does not operate at a constant and maximal rate Smith accepts the possibility that Tm^{d}_{Na} is subject to increase or decrease in response to one or several physiological factors (5) Another possibility as considered in the next paragraph is that with a decrease in GFR an increased percentage of the filtered load is reabsorbed proximally lowering the distally presented load below Tm^{d}_{Na} If this lowered percentage of proximal reabsorption begins to return toward normal before plasma sodium level shows a certainly demonstrable rise (we do not

know whether or not this occurs) the distal load may again rise above Tm^d, with increase in output

Dr Surtshin and I(6) recently investigated postural effects on tubular reabsorptive activity in normal human subjects in maximal water diuresis The assumptions are made that (a) proximal reabsorption is isosmotic and (b) distal water reabsorption has ceased Creatinine clearance was slightly lower on standing Since the percentage fall in water excretion on changing from lying to standing was considerably greater than the percentage fall in GFR it follows that the percentage of filtered water reabsorbed proximally was increased on standing Since proximal reabsorption is considered as isosmotic the percentage of filtered sodium reabsorbed proximally was also increased on standing Since free water clearance showed a greater percentage fall on standing than did the GFR distal reabsorption of solutes largely sodium salts showed a greater percentage fall than the GFR Thus we conclude that on changing from lying to standing in maximum water diuresis the percentage of filtered water and sodium reabsorbed proximally is increased while the percentage (and absolute amount per unit time) of sodium reabsorbed distally is decreased The same conclusions should follow from any combination of circumstances leading in maximum water diuresis to a greater percentage fall in urine flow and free water clearance than in GFR While maximum water diuresis is used here as a tool to determine proximal water and sodium reabsorption and distal sodium reabsorption we see no reason why the same considerations should not apply at other rates of urine flow If these interpretations are valid it is seen that considerable variations in the proportions of sodium reabsorbed proximally and distally can occur physiologically

Changes in ECF Volume No evidence is known for any type of stretch or baroreceptor in the interstitial spaces but they are known to exist in blood vessel walls in various regions If one is to postulate extracellular fluid volume effects regulating excretion of water and sodium it therefore seems most reasonable to postulate such stretch receptors in vessel walls Evidence that such stretch receptors operate in vessels of the head has been reported (7 8) while others have failed to obtain these results (9 10) Evidence for intrathoracic receptors presumably on the venous side of the pulmonary circuit affecting renal excretion of water without effect on sodium excretion or the GFR is reported by Gauer et al (11 12) We were unable to find any difference between the normal and the denervated kidneys responses to such stimulation(13) Since the increased urine flow occurs without a change in the GFR or the rate of sodium excretion and is unaffected by renal denervation a reflex inhibition of *ADH formation seems the most likely mechanism* We are inclined to discount the importance of such stretch receptors in regu

lating blood and interstitial fluid volume since the increased water output is not accompanied by sodium

Humoral Influences We will mention here only the well known effect of ADH in increasing distal reabsorption of water without solute and the increased reabsorption of sodium under the influence of various adrenal steroids particularly aldosterone The important but unanswered question of how the output of aldosterone is regulated can only be touched on here it may well prove of importance in the edemas of congestive failure and of the nephrotic syndrome

Dogs with demonstrated complete absence of neurohypophysis and median eminence can still form a hypertonic urine on water deprivation but the question must remain open as to whether the tubules in severely dehydrated subjects can still reabsorb some water in excess of solutes without ADH or whether the effect is due to residual formation of ADH in the hypothalamus

Reflex stimulation of ADH formation in response to painful stimuli or to fright has been demonstrated beyond a reasonable doubt(14) Various workers have reported hypnotically induced water diuresis in man presumably resulting from cerebral inhibition of ADH formation There are also reports that in dogs diuresis will occur without water administration even when the kidney is denervated if a conditioned stimulus has been established by repeated applications with water These findings are consistent with the view of reflex inhibition of ADH formation but Bykow s finding(15) that conditioned initiation or inhibition of water diuresis persists after hypophysectomy but not after renal denervation plus hypophysectomy is interpreted as indicating cortical control of water excretion at the renal level independently of variations in ADH formation Whether this direct renal effect is vascular or tubular remains unanswered even if one grants that it exists It may well be that Bykow s removal of ADH forming tissues was incomplete although this would not explain the lack of effect after renal denervation since the influence of ADH is still seen in the denervated kidney The entire matter of central nervous effects on water and sodium excretion remains in an unsatisfactory state although my personal view is that the evidence for direct nervous influences on tubular activity is quite inconclusive (15 16)

We may briefly consider some clinical evidence for central nervous system regulation of sodium and water excretion Salt wasting leading to severe hyponatremia and dehydration in the presence of apparently normal anterior pituitary and adrenal cortical function has been described (17 18) The ability of the tubules to conserve sodium is impaired even though plasma sodium level is low While no certain conclusion can be drawn as to its mechanism the condition apparently differs from the salt losing syndrome attributable to an inherent tubular defect in that it disappears on recovery from the central nervous lesion

In closing we may be permitted a few speculations although we are able only to restate questions without answering them We hall start from the idea which of cour e is not original that the *renal* role in edema formation is an exces ive tubular reabsorption of sodium (with accompanying water) in relation to the filtered load Taking congestive failure as an example we may conclude that there is not primary aberration of tubular function since this function returns to normal as cardiac function improves The que tion is What is re pon ible for this increa ed tubular activity? We may po tu late some humoral sub tance produced in excess in conge tive failure and deficient in the cerebral salt lo ing syndrome Can this be aldo terone? Aldosterone production is known to be but little reduced after hypophy sectomy although the output of other adrenal steroid is greatly reduced(19) This is consi tent with the finding that hypophy ectomized dogs can main tain normal or almo t normal sodium balance (20 21) While the princi pal factor immediately responsible for the regulation of aldo terone produc tion remains unknown apparently it is not the anterior pituitary

Aldosterone excretion and pre umably production is increa ed in alt deprivation and in edematous patients while expan ion of body fluids by water plus pitre sin in sodium depleted subjects decrea es the previously high aldo terone excretion and increa es odium excretion de pite hyponatremia suggesting a role of fluid volume change(22) When pitressin is stopped the retained water is eliminated aldo terone excretion rises and salt excretion drops the subject has had a net loss of alt There is increa ed excretion of a sodium retaining substance probably aldo terone in nephrosis and in heart failure when sodium excretion is low Excretion of the presumed aldosterone dimini hes during diure is when odium excretion increase It is not found in Addi on s disea e or after bilateral adrenalectomy Its ex cretion is increa ed on salt deprivation and dimini hed on high salt in take(23) The unexpectedly high output in sodium losing nephritis may simply mean that in this condition the tubules are in ensitive to its action

It appears justifiable to entertain the hypothesis that the reduction of alt excretion at least in salt deprivation in the normal subject is the re ult and not the cau e of increased aldosterone production On depriving a normal subject of salt or on extrarenal lo s of alt output exceeds intake and the resultant fall in plasma level or fluid volume or both call forth increa ed aldosterone production with resultant low urinary (and sweat) output of alt On the other hand if the kidneys are unable to conserve alt in a normal fashion perhaps becau e of in ensitivity to aldo terone negative sodium balance may persist even with high hormone production That fluid volume may be a more important factor than plasma sodium level is sugge ted by the finding that aldosterone excretion increased greatly within a few hours in a diabetes in ipidus subject deprived of water although a rise in plasma

sodium level is not excluded here. Normal men respond to relatively small increases in sodium load with a great decrease in urinary aldosterone but heart failure and portal cirrhosis subjects are much less responsive. Aldosterone secretion was decreased on increase in ECF volume and increased on decrease of ECF volume regardless of whether ECF osmolality rose, fell or was unchanged(24). Normal dogs excrete only traces of aldosterone while heart failure or caval constriction dogs excrete large amounts(25).

Speculation that hyperaldosteronism may be an important factor in the pathogenesis of cardiac or nephrotic edema must be tempered by Conn's finding(26) of primary hyperaldosteronism due to adrenocortical tumor, symptomatically relieved by potassium administration, cured by operation and characterized by hypertension, transitory paralyses, polyuria resistant to pitressin, absence of edema, high sodium and low potassium plasma levels and high sodium with low potassium in muscle. Kidney biopsy showed the tubular vacuolization and necrosis characteristic of severe potassium depletion. This is far from the picture of congestive failure. It may well represent, however, an extreme situation where the deleterious effects of potassium depletion are the principal manifestation. The kidney tubules have suffered such a derangement of function that the reabsorption of sodium and water cannot increase in the expected way in response to high aldosterone level.

Perhaps no one would maintain that hyperaldosteronism is a primary factor in cardiac or nephrotic edema. Some of us at least believe that the accumulation of these edemas depends principally on physical factors: during such accumulation output of salt and water necessarily falls below intake or, stated differently, the difference between filtered and reabsorbed sodium and water falls below the intake. We must admit that we do not know why reabsorption during this period is excessive relative to the filtered load, so it may still be permissible to wonder whether a humoral factor less marked than that seen in cases such as Conn's is operating. During steady state edema the subject is again in balance; it is not known why the relation between filtration and reabsorption has again changed so that output now equals intake, and the same considerations apply in the clearing of edema. Presumably a balance is achieved between the retaining effect of aldosterone and the various factors tending to promote excretion. We are convinced that mere reduction of filtration is not responsible for retention since with experimental chronic bilateral reduction of GFR in otherwise normal kidneys balance is reestablished before any demonstrable edema has developed.

It has been postulated that the hyperaldosteronism of the edemas is a secondary development. Not only is the mechanism triggering the increased hormone production unknown but the effect is opposite to that expected. Experimentally induced acute increase of fluid volume lead to diminished aldosterone and increased sodium excretion, the reverse of the findings in

clinical edema If there is any cau al relation the fluid volume changes in clinical edema mu t be the result and not the cause of the increase in aldo sterone For additional reference relating to the last five paragraphs se (27 28 29) Reference mu t al o be made to Selkurt s excellent review (30) of the general topic of odium and water excretion

To summarize deprivation of alt and water in normal subjects increa es aldosterone production while alt and water loads decrease it The primary event is a fall or ri e in FCF volume or odium level or both with the result ing rise or fall in aldosterone production helping the tubules to bring about a return to the normal tatus In heart failure on the other hand excess of FCF volume and of body sodium load appearing as edema is accompanied not by a fall as in the normal but by a ri e in aldo terone production And with the natriure is of clearing edema aldo terone production fall In such condition the changes in aldo terone production are not the re ult but one of the cau e of the fluid change if any cau al relation exi ts The cau e of such increa ed aldo terone production in spite of an exce s water and odium load remains unexplained

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